

WHAT IS CLAIMED IS:

1. A DNA analyzing method comprising preparing a single-stranded DNA fragment from a sample double-stranded DNA fragment, denaturing the conformation of the single-stranded DNA fragment under a given denaturing condition, preparing a melting curve data representing the relation between the denaturing condition and the denaturing results, and comparing the melting curve data with the melting curve data of the conformation of a single-stranded DNA fragment from a double-stranded DNA fragment of known DNA sequence when the conformation is denatured under a denaturing condition, wherein the sample double-stranded DNA fragment is represented on the basis of the relation thereof with the double-stranded DNA fragment of known DNA sequence from the comparison results.

2. A DNA analyzing method comprising preparing a single-stranded DNA fragment from a sample double-stranded DNA fragment, intercalating an intercalating agent capable of emitting fluorescence of a given wave length on receiving excitation beam of another given wave length with the base pairing formed in the complementary sequence of the conformation of the

single-stranded DNA fragment if such conformation is formed, irradiating the excitation beam of the given wave length onto the single-stranded DNA fragment intercalated with the intercalating agent, denaturing the conformation of the single-stranded DNA fragment under a given denaturing condition while irradiating the excitation beam, detecting the change in the intensity of the fluorescence of the given wave length due to the denaturing as the denaturing results, preparing a melting curve data representing the relation between the denaturing condition and the denaturing results, and comparing the melting curve data with the melting curve data of the conformation of a single-stranded DNA fragment from a double-stranded DNA fragment of known DNA sequence when the conformation is denatured under a denaturing condition, wherein the sample double-stranded DNA fragment is represented on the basis of the relation thereof with the double-stranded DNA fragment of known DNA sequence from the comparison results.

3. A DNA analyzing method according to claim 1, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting

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curve data comprises comparing the data of a freshly measured and input signal curve with one of the data sets of known template melting curves preliminarily prepared or with all of the data sets of the curves preliminarily prepared by linearly binding a plurality of the known template melting curves in combination, and determining that the data of a template curve with the least statistical error or the combination of the data sets of such template curves which in combination form a curve with the least statistical error is the sequence characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

4. A DNA analyzing method according to claim 2, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises comparing the data of a freshly measured and input signal curve with one of the data sets of known template melting curves preliminarily prepared or with all of the data sets of the curves preliminarily prepared by linearly binding a plurality of the known template melting curves in combination, and determining that the data of a template curve with

the least statistical error or the combination of the data sets of such template curves which in combination form a curve with the least statistical error is the sequence characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

5. A DNA analyzing method according to claim 1, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises calculating the statistical error between the data of a freshly measured and input signal curve and one of the data sets of known template melting curves preliminarily prepared or each of the data sets of the curves preliminarily prepared by linearly binding a plurality of the known template melting curves in combination, thereby selecting one curve data with the least error, carrying out the calculation and selection over each of the data sets of the known template melting curves or each of the curve data sets preliminarily prepared by linearly binding a different combination of the data sets of the known template melting curves, and representing a given number of the curve data selected from the group of all

of the data sets of the curves in the increasing order of the statistical error as the sequence characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

6. A DNA analyzing method according to claim 2, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises calculating the statistical error between the data of a freshly measured and input signal curve and one of the data sets of known template melting curves preliminarily prepared or each of the data sets of the curves preliminarily prepared by linearly binding a plurality of the known template melting curves in combination, thereby selecting one curve data with the least error, carrying out the calculation and selection over each of the data sets of the known template melting curves or each of the curve data sets preliminarily prepared by linearly binding a different combination of the data sets of the known template melting curves, and representing a given number of the curve data selected from the group of all of the data sets of the curves in the increasing order of the statistical error as the sequence

characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

7. A DNA analyzing method according to claim 1, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the absorbance of a sample via temperature.

8. A DNA analyzing method according to claim 2, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the fluorescence intensity of a sample via temperature.

9. A DNA analyzing method according to claim 3, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the absorbance of a sample via temperature.

10. A DNA analyzing method according to claim 4, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the fluorescence intensity of a sample via temperature.

11. A DNA analyzing method according to claim 1,

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wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in absorbance depending on the alternative changing.

12. A DNA analyzing method according to claim 2, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in the fluorescence intensity.

13. A DNA analyzing method according to claim 3, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in absorbance depending

on the alternative changing.

14. A DNA analyzing method according to claim 5, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the absorbance of a sample via temperature.

15. A DNA analyzing method according to claim 5, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in absorbance depending on the alternative changing.

16. A DNA analyzing method according to claim 4, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in the fluorescence intensity depending on the alternative changing.



17. A DNA analyzing method according to claim 6, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the fluorescence intensity of a sample via temperature.

18. A DNA analyzing method according to claim 6, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in the fluorescence intensity depending on the alternative changing.

19. A DNA analyzing method according to claim 2, wherein the intercalating agent is ethidium bromide.

20. A DNA analyzer comprising;  
a holding means holding a sample solution containing one type of single-stranded DNA fragment or plural types of single-stranded DNA fragments;  
a spectroscopic means measuring the UV absorbance of the sample solution containing said single-stranded

DNA fragment(s);

a denaturing means having an action depending on the given denaturing condition onto the sample solution so as to denature the conformation formed by the single-stranded fragment(s) under given conditions and a denaturing condition regulatory means regulating the denaturing condition; and

a signal processing means for inputting the signals for the regulation of the denaturing condition and the signals for the spectroscopic measurement which are then saved therein for processing,

wherein by changing the denaturing condition of the conformation of the single-stranded DNA fragment(s) by the denaturing condition regulatory means to prepare the melting curve data of the single-stranded DNA fragment sample over the change in the denaturing condition and subsequently comparing the melting curve data with the melting curve data of the conformation of a single-stranded DNA fragment from a double-stranded DNA fragment of known DNA sequence when the conformation is denatured under a denaturing condition, the sample double-stranded DNA fragment is represented on the basis of the relation thereof with the double-stranded DNA fragment of known DNA sequence from the

comparison results.

21. A DNA analyzer comprising;

a holding means holding a sample solution containing one type of single-stranded DNA fragment or plural types of single-stranded DNA fragments;

a means for intercalating an intercalating agent capable of emitting fluorescence of a given wave length on receiving excitation beam of another given wave length with the base pairing formed in the complementary sequence forming the conformation of a single-stranded DNA fragment(s) in the sample solution; and

a means for irradiating excitation beam of the given wave length onto the single-stranded DNA fragment(s) intercalated with the intercalating agent,

wherein by changing the denaturing condition of the conformation of the single-stranded DNA fragment(s) under the irradiation of the excitation beam to prepare the melting curve data of the single-stranded DNA fragment sample over the change in the denaturing condition, and subsequently comparing the melting curve data with the melting curve data of the conformation of

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a single-stranded DNA fragment from a double-stranded DNA fragment of known DNA sequence when the conformation is denatured under a denaturing condition, the sample double-stranded DNA fragment is represented on the basis of the relation thereof with the double-stranded DNA fragment of known DNA sequence from the comparison results.

22. A DNA analyzer according to claim 20, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises comparing the data of a freshly measured and input signal curve with one of the data sets of known melting curves preliminarily prepared or with all of the combinations of the curve data sets preliminarily prepared by linearly binding a plurality of template curve data sets, and determining that the data of a template curve with the least statistical error or the combination of the data sets of such template curves which in combination form a curve with the least statistical error is the sequence characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

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23. A DNA analyzer according to claim 21, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises comparing the data of a freshly measured and input signal curve with one of the data sets of known melting curves preliminarily prepared or with all of the combinations of the curve data sets preliminarily prepared by linearly binding a plurality of template curve data sets, and determining that the data of a template curve with the least statistical error or the combination of the data sets of such template curves which in combination form a curve with the least statistical error is the sequence characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

24. A DNA analyzer according to claim 20, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises calculating the statistical error between the data of a freshly measured and input signal curve and one of the data sets of known template melting curves preliminarily prepared or each of the data sets of the curves preliminarily prepared by

linearly binding a plurality of the known template melting curves in combination, thereby selecting one curve data with the least error, carrying out the calculation and selection over each of the remaining data sets of the known template melting curves or each of the data sets of the curves preliminarily prepared by linearly binding a plurality of the known template melting curves in combination, and representing a given number of the selected curve data in the increasing order of the statistical error as the sequence characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

25. A DNA analyzer according to claim 21, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises calculating the statistical error between the data of a freshly measured and input signal curve and one of the data sets of known template melting curves preliminarily prepared or each of the data sets of the curves preliminarily prepared by linearly binding a plurality of the known template melting curves in combination, thereby selecting one curve data with the least error, carrying out the

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calculation and selection over each of the remaining data sets of the known template melting curves or each of the data sets of the curves preliminarily prepared by linearly binding a plurality of the known template melting curves in combination, and representing a given number of the selected curve data in the increasing order of the statistical error as the sequence characteristics of the measured single-stranded DNA fragment from the sample double-stranded DNA fragment.

26. A DNA analyzer according to claim 20, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the absorbance of a sample via temperature.

27. A DNA analyzer according to claim 21, wherein the denaturing condition is temperature and the data of a melting curve is derived from the change in the fluorescence intensity of a sample via temperature.

28. A DNA analyzer according to claim 22, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the absorbance of a sample via temperature.

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29. A DNA analyzer according to claim 23, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the fluorescence intensity of a sample via temperature.

30. A DNA analyzer according to claim 20, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in absorbance depending on the alternative changing.

31. A DNA analyzer according to claim 21, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in the fluorescence intensity depending on the alternative changing.

32. A DNA analyzer according to claim 22, wherein



the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in absorbance depending on the alternative changing.

33. A DNA analyzer according to claim 24, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the absorbance of a sample via temperature.

34. A DNA analyzer according to claim 24, wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in absorbance depending on the alternative changing.

35. A DNA analyzer according to claim 23, wherein the sequence information of a single-stranded DNA

fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in the fluorescence intensity depending on the alternative changing.

36. A DNA analyzer according to claim 25, wherein the denaturing condition is temperature and the melting curve data is derived from the change in the fluorescence intensity of a sample via temperature.

37. A DNA analyzer according to claim 25 wherein the sequence information of a single-stranded DNA fragment is obtained by alternatively changing the denaturing condition for the progress in denaturing and for the formation of the conformation in a continuous manner, and measuring and analyzing the hysteresis characteristics of the change in the fluorescence intensity depending on the alternative changing.

38. A DNA analyzer according to claim 21, wherein the intercalating agent is ethidium bromide.

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39. A DNA analyzer comprising;

an enzymatic reaction means for effecting the selective amplification of a specific DNA region and simultaneously producing a single-stranded DNA fragment as an analytical subject;

a holding means holding a sample solution containing the single-stranded DNA fragment;

a spectroscopic means measuring the UV absorbance of the sample solution containing said single-stranded DNA fragment, a denaturing means having an action depending on the given denaturing condition onto the sample solution so as to denature the conformation formed by the single-stranded fragment under given conditions and a denaturing condition regulatory means regulating the denaturing condition; and

a signal processing means for inputting the signals for the regulation of the denaturing condition and the signals for spectroscopic measurement which are then saved therein for processing,

wherein by preparing the melting curve data of the single-stranded DNA fragment sample over the change in the denaturing conditions for the conformation of the single-stranded DNA fragment with the denaturing condition regulatory means, and subsequently comparing

the melting curve data with the melting curve data of the conformation of a single-stranded DNA fragment from a double-stranded DNA fragment of known DNA sequence when the conformation is denatured under a denaturing condition, the sample double-stranded DNA fragment is represented on the basis of the relation thereof with the double-stranded DNA fragment of known DNA sequence from the comparison results.

40. A DNA analyzer according to claim 39, wherein the single-stranded DNA fragment is a specific single-stranded DNA fragment prepared from a specific DNA region fragment preliminarily amplified and enriched by PCR in a manner specific to the region.

41. A DNA analyzer according to claim 40, wherein the single-stranded DNA fragment is a specific single-stranded DNA fragment preliminarily amplified in a manner specific to the region by asymmetric PCR capable of replicating an excess amount of the objective single-stranded DNA fragment by setting the volume ratio of a pair of primers to be used for PCR at an uneven ratio.

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42. A DNA analyzer according to claim 40, wherein the single-stranded DNA fragment is a specific single-stranded DNA fragment prepared by removing either one of a single-stranded DNA fragment not immobilized on a support or a single-stranded DNA fragment immobilized on the support from the PCR products amplified with one primer preliminarily immobilized on the support or the other primer not immobilized on the support.

43. A DNA analyzing method according to claim 1, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises comparing the data of a freshly measured and input signal curve as the defined combination of given functions with known template melting curve data preliminarily prepared as the defined combination of given functions and

determining that a template curve data with the least statistical error is defined as the sequence characteristics of the measured single-stranded DNA fragment of the sample double-stranded DNA fragment.

44. A DNA analyzing method according to claim 2, wherein the comparison of the melting curve data of the

sample double-stranded DNA fragment with known melting curve data comprises comparing the data of a freshly measured and input signal curve as the defined combination of given functions with known template melting curve data preliminarily prepared as the defined combination of given functions, and determining that a template curve data with the least statistical error is defined as the sequence characteristics of the measured single-stranded DNA fragment of the sample double-stranded DNA fragment.

45. A DNA analyzing method according to claim 21, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises comparing the data of a freshly measured and input signal curve as the defined combination of given functions with known template melting curve data preliminarily prepared as the defined combination of given functions, and determining that a template curve data with the least statistical error is defined as the sequence characteristics of the measured single-stranded DNA fragment of the sample double-stranded DNA fragment.

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46. A DNA analyzing method according to claim 22, wherein the comparison of the melting curve data of the sample double-stranded DNA fragment with known melting curve data comprises comparing the data of a freshly measured and input signal curve as the defined combination of given functions with known template melting curve data preliminarily prepared as the defined combination of given functions, and determining that a template curve data with the least statistical error is defined as the sequence characteristics of the measured single-stranded DNA fragment of the sample double-stranded DNA fragment.

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